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Linezolid Alone or Combined with Rifampin against Methicillin-Resistant *Staphylococcus aureus* in Experimental Foreign-Body Infection▽

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We investigated the activity of linezolid, alone and in combination with rifampin (rifampicin), against a methicillin-resistant *Staphylococcus aureus* (MRSA) strain in vitro and in a guinea pig model of foreign-body infection. The MIC, minimal bactericidal concentration (MBC) in logarithmic phase, and MBC in stationary growth phase were 2.5, >20, and >20 μg/ml, respectively, for linezolid; 0.01, 0.08, and 2.5 μg/ml, respectively, for rifampin; and 0.16, 0.63, >20 μg/ml, respectively, for levofloxacin. In time-kill studies, bacterial regrowth and the development of rifampin resistance were observed after 24 h with rifampin alone at 1× or 4× the MIC and were prevented by the addition of linezolid. After the administration of single intraperitoneal doses of 25, 50, and 75 mg/kg of body weight, linezolid peak concentrations of 6.8, 12.7, and 18.1 μg/ml, respectively, were achieved in sterile cage fluid at ≈3 h. The linezolid concentration remained above the MIC of the test organism for 12 h with all doses. Antimicrobial treatments of animals with cage implant infections were given twice daily for 4 days. Linezolid alone at 25, 50, and 75 mg/kg reduced the planktonic bacteria in cage fluid during treatment by 1.2 to 1.7 $\log_{10}$ CFU/ml; only linezolid at 75 mg/kg prevented bacterial regrowth 5 days after the end of treatment. Linezolid used in combination with rifampin (12.5 mg/kg) was more effective than linezolid used as monotherapy, reducing the planktonic bacteria by ≥3 $\log_{10}$ CFU ($P < 0.05$). Efficacy in the eradication of cage-associated infection was achieved only when linezolid was combined with rifampin, with cure rates being between 50% and 60%, whereas the levofloxacin-rifampin combination demonstrated the highest cure rate (91%) against the strain tested. The linezolid-rifampin combination is a treatment option for implant-associated infections caused by quinolone-resistant MRSA.

Implanted devices are increasingly used in modern medicine to alleviate pain or improve a compromised function. Implant-associated infections represent an emerging complication caused by organisms which adhere to the implant surface and grow embedded in a protective extracellular polymeric matrix, known as a biofilm (7, 8, 41). In addition, the microorganisms in biofilms enter a stationary growth phase and become phenotypically resistant to most antimicrobials, frequently causing treatment failure. In such cases, surgical removal of the implant is often required, causing high morbidity and substantial health care costs (5, 14, 32).

Staphylococcus aureus is the most common pathogen causing implant-associated infections (5, 7). Successful treatment of these infections includes early surgical intervention and antimicrobial treatment with bactericidal drugs that also act on surface-adhering microorganisms. Rifampin (rifampicin) is bactericidal against stationary-growth-phase staphylococci, as demonstrated in vitro, in experimental animal models, and in clinical studies (9, 43). However, when it is used as a single agent, the rapid emergence of rifampin resistance occurs (37). Therefore, the use of antimicrobial combinations to prevent the development of rifampin resistance during treatment have been investigated (34, 36, 39). Rifampin in combination with quinolones has successfully been used for the treatment of orthopedic implant-related infections (9, 25, 35). However, the increasing prevalence of quinolone-resistant staphylococci has urged investigations for alternative drugs for use in combination with rifampin treatment (4, 30). In particular, methicillin-resistant staphylococci represent an increasing challenge due to their resistance to a broad variety of antimicrobials (23, 33).

The oxazolidinone linezolid is active against gram-positive cocci, including methicillin-resistant *Staphylococcus aureus* (MRSA) (11, 20, 24, 28). Limited data on the use of the linezolid-rifampin combination for the treatment of implant-associated MRSA infections are available. In vitro time-kill experiments showed a potential additive effect between linezolid and rifampin against MRSA (12). However, only case reports or small case series describing the treatment of implant-associated infections with linezolid and rifampin exist (2, 16, 19, 26).

In the study described here, we investigated the activity of linezolid, alone and in combination with rifampin, against one reference MRSA strain in vitro and in an established foreign-body infection model. The cage-associated infection model in guinea pigs has been validated for testing the activities of antimicrobial agents and their combinations against implant-associated infections in preclinical studies (38, 42).

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MATERIALS AND METHODS

Study organism. MRSA strain ATCC 43300, which is susceptible to levofloxacin and rifampin, was used for in vitro and in vivo antimicrobial testing. Methicillin-susceptible *S. aureus* strain ATCC 29213 was used as the indicator organism for the agar diffusion bioassay. The strains were stored at −70°C by use of a cryovial bead preservation system (Microbank; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). One cryovial bead was cultured overnight on Columbia sheep blood agar plates (Becton Dickinson, Heidelberg, Germany). Inocula were prepared from subcultures of two to three colonies, which were resuspended in 5 ml of Trypticase soy broth (TSB) and incubated overnight at 37°C without shaking.

Antimicrobial agents. Linezolid was provided as a purified powder by the manufacturer (Pfizer AG, Zurich, Switzerland); stock solutions of 2.5 mg/ml were prepared in sterile pyrogen-free water. Levofloxacin hemihydrate injectable solution (5 mg/ml; Aventis Pharma AG, Zurich, Switzerland) and rifampin (Sandoz AG, Steinhausen, Switzerland) were purchased from the respective manufacturers.

In vitro antimicrobial susceptibility. The in vitro susceptibility of the MRSA strain to linezolid, levofloxacin, and rifampin was determined in triplicate by using a standard inoculum of 1×10^5 to 5×10^5 CFU/ml, adjusted from overnight cultures. The MIC was determined in Mueller-Hinton broth (MHB) by the macrodilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) (6). In brief, 10 twofold serial dilutions of the test drug were prepared in 2 ml MHB in sterile borosilicate glass tubes. Two milliliters of the antimicrobial dilutions was inoculated below the meniscus, and the tubes were incubated for 18 h at 37°C without shaking. The MIC was the lowest drug concentration that inhibited visible bacterial growth. Tubes without visible growth were then vigorously vortexed, incubated for 4 h at 37°C without shaking, and assessed for viable bacteria by plating the contents of the tubes on agar. The lowest antimicrobial concentration which killed ≥99.9% of the initial bacterial count (i.e., ≥3 $\log_{10}$ CFU/ml) was defined as the minimum bactericidal concentration (MBC) during logarithmic growth (MBC_{log}), as described in the *Manual of Clinical Microbiology* (18). The killing of bacteria during stationary growth phase (MBC_{stat}) was assayed in nutrient-restricted medium (0.01 M phosphate-buffered saline, pH 7.4), as described previously (39). In this medium, bacterial counts remained within ±15% of the initial inoculum in the antimicrobial-free culture for >36 h.

In vitro time-kill studies. The antimicrobial activities of linezolid and rifampin, alone and in combination, against the MRSA strain were evaluated by time-kill studies with inocula of 1×10^6 to 5×10^6 CFU/ml, as described previously (15). Antibiotic solutions with 1× and 4× the MIC of the test strain were prepared in 10 ml of MHB. Growth in the absence of antibiotics served as the control. Colony counts were determined after 0, 6, and 24 h of incubation at 37°C by plating aliquots of appropriate dilutions on Mueller-Hinton agar (MHA). The ≥10-fold dilutions allowed accurate colony counts in the range of 10 to 250 CFU per plate and minimized the effects of drug carryover. The quantification limit was set equal to 200 CFU/ml (>10 CFU in 50 μl of a 10-fold dilution). Killing over time was expressed as the mean reduction in the $\log_{10}$ CFU/ml ± the standard deviation (SD). Synergism was defined as a 100-fold increase in the level of killing at 24 h with the combination in comparison with the level of killing achieved with the most active single drug. Antagonism was defined as a 100-fold decrease in the level of killing at 24 h with the combination of both drugs compared to the level of killing achieved with the most active single drug (15). Cultures of the MRSA strain that were exposed to rifampin alone or in combination with linezolid and that showed visible growth after 24 h of incubation were tested for rifampin resistance. The cultures were adjusted to a standardized inoculum corresponding to a McFarland 0.5 standard, spread on MHA containing rifampin (1 μg/ml), and assessed for growth. Experiments were performed in triplicate.

In vitro antimicrobial resistance studies. An assay was developed to evaluate the rate of in vitro emergence of rifampin resistance. The ratio of resistant to total colony counts was assessed after 24 h of incubation of the MRSA strain in 10 ml MHB containing rifampin alone or rifampin and linezolid at 1× the MIC. The 24-h bacterial cultures were serially diluted 10-fold, 50-μl aliquots were plated on MHA containing rifampin (1 μg/ml) or no antibiotic, and the colonies were counted after 48 h of incubation at 37°C. The results were expressed as a ratio between the rifampin-resistant $\log_{10}$ CFU/ml and the total $\log_{10}$ CFU/ml. Experiments were performed in triplicate.

Animal model. A foreign-body infection model in guinea pigs was used, as described previously (3, 38, 40, 42). Guinea pigs were kept under specific-pathogen-free conditions in the Animal House of the Department of Biomedicine, University Hospital Basel, and animal experimentation guidelines according to the regulations of Swiss veterinary law were followed. The study protocol was approved by the Institutional Animal Care and Use Committee. In brief, four sterile polytetrafluoroethylene (Teflon) cages (32 mm by 10 mm) perforated with 130 regularly spaced holes of 1 mm in diameter (Angst-Pfister AG, Zurich, Switzerland) were subcutaneously implanted in the flanks of male albino guinea pigs (Charles River, Sulzfeld, Germany) under aseptic conditions. Animals weighing 550 to 600 g were anesthetized with an intramuscular injection of ketamine (20 mg/kg of body weight) and xylazine (4 mg/kg). Two weeks after surgery and healing of the surgical wounds, the sterility of the cages was verified by culture of the aspirated cage fluid. Contaminated cages were excluded from further studies. Sterile cages were used for the pharmacokinetic studies. For the treatment studies, the cages were infected by percutaneous inoculation of 200 μl containing 2×10^4 CFU of the MRSA strain (day 0). Before inoculation, overnight bacterial cultures were washed twice, resuspended in 5 ml of sterile pyrogen-free normal saline, and diluted 1:1,000. The establishment of infection was confirmed 24 h later by quantitative culture of aspirated cage fluid.

Pharmacokinetic studies. Cage fluid was aspirated from noninfected animals over 24 h (1, 2, 4, 6, 8, 10, 12, and 24 h) following intraperitoneal administration of a single dose of linezolid at 25, 50, and 75 mg/kg. Each dose was tested in three guinea pigs; therefore, 12 cages were used to relate the pharmacokinetic parameters to the antimicrobial treatment efficacy results. At each time point, 150-μl aliquots of cage fluid were aspirated from two cages from each animal (i.e., six replicates per time point and drug dose). The collected fluid was centrifuged ($2{,}100 \times g$ for 7 min), and the supernatant was stored at −20°C until further analysis.

Determination of drug concentrations. Linezolid concentrations in cage fluid were determined by an agar plate diffusion bioassay with *S. aureus* strain ATCC 29213 as the indicator organism. Antibiotic medium 1 (Difco, BD, Le Pont de Claix, France) was suspended in sterile pyrogen-free water, and the mixture was boiled at 100°C in a water bath for 30 min. After the medium was boiled, it was cooled to 50°C, inoculated with the overnight culture of the indicator organism (300 μl/400 ml medium), and poured into large assay plates (30 by 30 cm). Calibration curves were plotted for each of the assay plates, and the regression fitting equation was extrapolated. The standard solutions were prepared in 31% guinea pig serum (corresponding to the linezolid-albumin binding ratio in humans) by preparing twofold serial dilutions of the 20-mg/liter linezolid solution (27). One hundred microliters of the cage fluid samples and duplicates of the linezolid standard solutions were spotted into holes punched into the assay plates, and the plates were incubated overnight. The diameter of the inhibition zone was measured with calipers. The bioassay detection limit corresponded to the linezolid MIC of the indicator organism (i.e., 1.25 μg/ml).

Pharmacokinetic parameters. The concentration-time data were analyzed individually for each animal by using the WinNonlin software package (Pharsight Corp., Mountain View, CA). Mean ± SD values of the peak concentration (C_{max}), the time to reach C_{max} (T_{max}), the trough concentration at 12 h after dosing (C_{min}), half-life ($t_{1/2}$), and the area under the concentration-time curve from time zero to 24 h (AUC_{0-24}) were calculated from three animals receiving the same linezolid dose.

Antimicrobial treatment studies. Antimicrobial treatment was initiated 24 h after infection (day 1). At least three animals were randomized into each of the following treatment groups: control (saline); linezolid at 25, 50, and 75 mg/kg (alone or in combination with rifampin at 12.5 mg/kg); and levofloxacin at 10 mg/kg in combination with rifampin at 12.5 mg/kg (22). All antibiotics were administered intraperitoneally every 12 h over 4 days (i.e., a total of eight doses).

Efficacy of treatment against planktonic bacteria. The planktonic bacteria in the aspirated cage fluid were enumerated before the initiation of antimicrobial treatment (day 1), on the fourth day of treatment and before administration of the last antimicrobial dose (day 4), and 5 days after the end of treatment (day 10). Bacterial counts were expressed as the median and interquartile range of the $\log_{10}$ CFU/ml. The quantification limit of the planktonic bacteria was set at 1,000 CFU/ml (>10 CFU in 50 μl from dilutions ≥10-fold). Thus, negative cage fluid cultures were assigned a value of 3 $\log_{10}$ CFU/ml for calculation of the $\log_{10}$ CFU/ml reduction and for statistical analysis. The efficacy of the treatment against planktonic bacteria was expressed as (i) the difference in bacterial counts in cage fluid ($\Delta\log_{10}$ CFU/ml = $\log_{10}$ CFU/ml [day 4 or 10] − $\log_{10}$ CFU/ml [day 1]) and (ii) the rate of culture-negative cage fluid samples, i.e., the number of cage fluid samples without the detectable growth of the MRSA strain divided by the total number of cages in the treatment group.

TABLE 1. In vitro susceptibility of MRSA strain ATCC 43300

Antibiotic	MIC (μg/ml)	MBC_{log} (μg/ml)	MBC_{stat} (μg/ml)
Linezolid	2.5	>20	>20
Rifampin	0.01	0.08	2.5
Levofloxacin	0.16	0.63	>20

Efficacy of treatment against adherent bacteria. To determine the efficacy of the treatment against adherent bacteria, the animals were sacrificed on day 10. The cages were removed under aseptic conditions, placed in 5 ml TSB, vortexed for 30 s, and incubated at 37°C. After 48 h, 100-μl aliquots of the cage cultures were plated on Columbia sheep blood agar plates (Becton Dickinson) and assessed for bacterial growth. Cultures displaying growth were tested by the *S. aureus* latex test (Staphytect Plus; Oxoid, Basel, Switzerland). Cage cultures negative by this test were considered contaminated and were not used for the evaluation of treatment efficacy. The efficacy of the treatment against adherent bacteria was expressed as the cure rate, defined as the number of cage cultures without MRSA growth divided by the total number of cages in the treatment group.

In vivo antimicrobial resistance studies. MRSA isolates from positive cultures on TSB containing the explanted cages (i.e., treatment failures) were screened for the in vivo development of rifampin resistance. For this purpose, multiple colonies of each morphologically distinct colony type were collected from an agar subculture, suspended in saline to a standardized inoculum corresponding to the turbidity of a McFarland 0.5 standard, and plated on MHA containing 1 μg/ml rifampin. The plates were incubated at 37°C and screened for growth after 48 h.

Statistics. Comparisons were performed by using the Mann-Whitney U test for continuous variables and a two-sided χ^2 or Fisher's exact test for categorical variables, as appropriate. For all tests, differences were considered significant when P values were <0.05. Figures were plotted with GraphPad Prism (version 4.0) software (GraphPad Software, La Jolla, CA).

RESULTS

In vitro antimicrobial susceptibility. Table 1 shows the in vitro susceptibility of the MRSA strain to linezolid, rifampin, and levofloxacin. Linezolid inhibited bacterial growth at 2.5 μg/ml, whereas a bactericidal effect was not achieved up to 20 μg/ml either in the logarithmic growth phase or in the stationary growth phase. Rifampin exerted a low MIC (0.01 μg/ml) and was bactericidal in the logarithmic and the stationary growth phases (MBC_{log} and MBC_{stat}, 0.08 and 2.5 μg/ml, respectively). Levofloxacin had a MIC of 0.16 μg/ml and exhibited bactericidal activity only against bacteria in the logarithmic growth phase (MBC_{log}, 0.63 μg/ml) and not those in the stationary phase (MBC_{stat}, >20 μg/ml).

In vitro time-kill studies. In vitro time-kill studies were performed with inocula of 1×10^6 to 5×10^6 CFU/ml to investigate the synergism or antagonism of linezolid and rifampin. In the controls, the bacterial counts increased by 1.7 $\log_{10}$ CFU/ml after 24 h. In the presence of linezolid at 1× the MIC, the bacterial counts remained unchanged, while at 4× MIC they decreased by 1.7 $\log_{10}$ CFU/ml at 24 h. Rifampin at both 1× and 4× the MIC similarly decreased the bacterial counts after 6 h (0.5 $\log_{10}$ CFU/ml); however, regrowth to counts similar to those for the growth controls occurred after 24 h (Fig. 1). Bacteria exposed to rifampin alone showed regrowth after 24 h and were resistant to rifampin. When rifampin was combined with linezolid at either 1× or 4× the MIC, the bacterial counts at 24 h were decreased by 1.6 and 1.8 $\log_{10}$ CFU/ml, respectively. Due to the development of rifampin resistance during exposure to rifampin alone, it was not possible to evaluate whether a potential synergistic or antagonistic interaction between rifampin and linezolid existed, as described above.

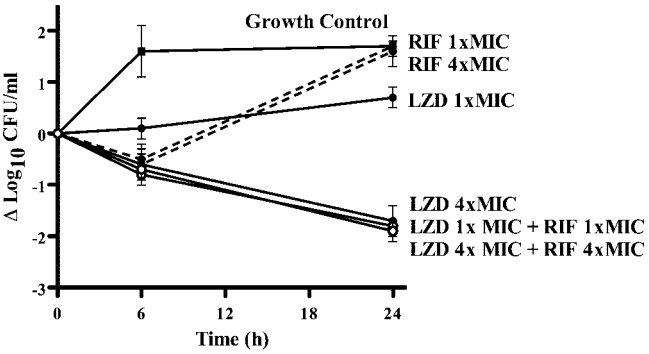

FIG. 1. Time-kill curves for 1× and 4× the MIC of linezolid (closed circles), 1× and 4× the MIC of rifampin (closed circle, dashed line), and their combination (open circles) against MRSA. Values are means ± SDs. LZD, linezolid; RIF, rifampin.

In vitro antimicrobial resistance. With an MRSA inocula of 1×10^6 to 5×10^6 CFU/ml, 94% ± 3% of total colony counts developed rifampin resistance 24 h after exposure to rifampin alone at 1× the MIC. In contrast, no rifampin-resistant colonies were detected after 24 h incubation with the rifampin-linezolid combination at 1× the MIC.

Pharmacokinetic studies. Figure 2 shows the concentration-time profile in cage fluid after the administration of a single intraperitoneal dose in noninfected animals. The calculated values of the pharmacokinetic parameters are summarized in Table 2. The C_{max}s of linezolid after the administration of a single intraperitoneal dose of 25, 50, or 75 mg/kg were 6.8, 12.6, and 18.1 μg/ml, respectively, which were achieved at ≈3 h after dosing. The linezolid concentration remained above the MIC of the test organism for 12 h (C_{min}, 2.8 to 3.3 μg/ml), as did the rifampin and levofloxacin concentrations (C_{min}s, 0.14 μg/ml and 0.27 μg/ml, respectively). The C_{max} of rifampin in the cage fluid reached almost 100× the MIC (C_{max}, 0.98 μg/ml), whereas this ratio was considerably lower for linezolid and levofloxacin (5× and 9× the MIC, respectively).

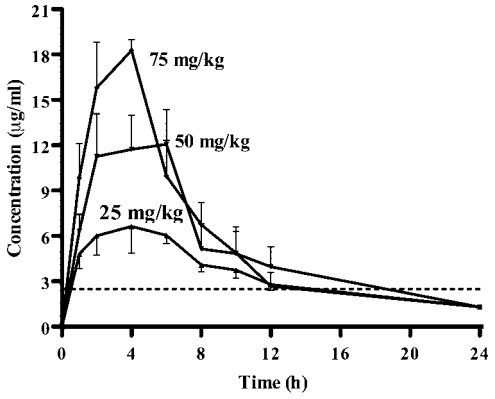

FIG. 2. Pharmacokinetics of linezolid in cage fluid after the administration of single intraperitoneal doses of 25 mg/kg, 50 mg/kg, and 75 mg/kg. The mean values of six measurements at each time point are shown; error bars represent SDs. The horizontal dotted line indicates the MIC of linezolid for the MRSA test strain.

TABLE 2. Values of pharmacokinetic parameters for the drugs tested in cage fluid after administration of a single intraperitoneal dose to noninfected animals

Antibiotic	Dose (mg/kg)	C_{max} (μg/ml)[a]	C_{min} (μg/ml)[a]	T_{max} (h)[a]	$t_{1/2}$ (h)[a]	AUC_{0-24} (μg · h/ml)[a]	C_{max}[b]/MIC	AUC_{0-24}[b]/MIC (h)
Linezolid	25	6.8 ± 1.3	3.0 ± 0.2	3.0 ± 0.4	6.8 ± 1.7	87.8 ± 2.7	2.7	35.1
Linezolid	50	12.7 ± 2.2	3.7 ± 1.1	3.3 ± 0.2	3.5 ± 1.7	118.7 ± 23.0	5.1	47.5
Linezolid	75	18.1 ± 1.7	2.5 ± 1.0	2.8 ± 0.2	2.6 ± 0.9	125.8 ± 20.5	7.2	50.3
Rifampin	12.5	1.0 ± 0.3	0.1 ± 0.1	2.1 ± 0.3	2.5 ± 1.3	4.6 ± 0.5	100.0	460
Levofloxacin	10	1.5 ± 0.2	0.3 ± 0.1	2.5 ± 0.3	4.2 ± 1.4	6.1 ± 0.8	9.4	38.1

[a] Values are means ± SDs from three animals, as predicted by use of the WinNonlin software package.
[b] The mean values of C_{max} and AUC_{0-24} in cage fluid after administration of a single intraperitoneal dose were used. Pharmacokinetic data for rifampin and levofloxacin were described previously (22, 31).

Antimicrobial treatment studies. Cage fluid sterility was confirmed prior to infection. At 24 h after infection, the median concentration of the bacteria enumerated in the cage fluid was 6.5 $\log_{10}$ CFU/ml. In control animals receiving saline, the bacterial counts in the cage fluid were 7.1 and 7.9 $\log_{10}$ CFU/ml after 4 and 10 days, respectively, which correspond to increases of 0.6 and 1.4 $\log_{10}$ CFU/ml, respectively. No spontaneous cure of the cage-associated infection occurred in the untreated animals.

Efficacy of treatment against planktonic bacteria. Table 3 shows the counts of planktonic bacteria and the rates of culture-negative cage fluid samples during and after treatment. During treatment (day 4), the bacterial counts in the cage fluid of animals treated with linezolid alone at 25, 50, and 75 mg/kg were decreased by median values of 1.4, 1.2, and 1.7 $\log_{10}$ CFU/ml, respectively. No differences in treatment efficacy were observed between the three linezolid doses ($P > 0.05$). Linezolid achieved culture negativity in 8% of the cage fluid samples when it was used at 25 mg/kg and 17% of the cage fluid samples when it was used at 50 and 75 mg/kg. When the three linezolid regimens were combined with rifampin, they reduced the bacterial counts by >3.0 $\log_{10}$ CFU/ml, which was significantly better than the results achieved with linezolid alone ($P < 0.05$) (Fig. 3A). A total of 55% to 65% of the cage fluid samples from animals treated with rifampin-linezolid combinations were culture negative on day 4.

Five days after the end of treatment (day 10), the planktonic bacteria in the cage fluid of animals treated with linezolid at 25 and 50 mg/kg showed regrowth to 7.3 and 7.1 $\log_{10}$ CFU/ml, respectively (Table 3), which correspond to increases of 1.0 and 0.8 $\log_{10}$ CFU/ml compared to the level of growth on day 1 (Fig. 3B). Linezolid at 75 mg/kg prevented bacterial regrowth in cage fluid on day 10, and the bacterial counts remained comparable to the values on day 4. In animals treated with the combination of linezolid and rifampin, the bacterial counts remained at the levels measured on day 4, independent of the linezolid dose ($P > 0.05$). No differences in treatment efficacy were observed between the three linezolid doses ($P > 0.05$) when they were combined with rifampin. The cure rates for animals treated with the linezolid-rifampin combination ranged from 75% to 95%, and rifampin resistance did not emerge.

Efficacy of treatment against adherent bacteria. No cure of cage-associated infections was observed with linezolid alone (Fig. 4). The use of linezolid in combination with rifampin showed cure rates of 50% to 60%. All linezolid-rifampin combinations exhibited significantly better activities than linezolid alone against adherent bacteria ($P < 0.001$). For comparison, the efficacy of the combination levofloxacin plus rifampin was tested and demonstrated a cure rate of 91%.

In vivo antimicrobial resistance studies. No rifampin-resistant MRSA strains were detected within positive cultures of cages from animals treated with rifampin alone or in combination with linezolid.

DISCUSSION

In this study, we investigated the activity of linezolid alone and in combination with rifampin against MRSA in vitro and in a guinea pig implant-associated infection model. The test

TABLE 3. Counts of planktonic bacteria in cage fluid and rate of culture-negative cage fluid samples during treatment (day 4) and 5 days after end of treatment (day 10)

Treatment group, dose (mg/kg)	Bacterial counts in cage fluid ($\log_{10}$ CFU/ml)[a]		No. of culture-negative cage fluid samples/total no. of samples (%)	
	Day 4	Day 10	Day 4	Day 10
Control (12)[b]	7.1 (7.0–7.5)	7.9 (7.6–8.1)	0/12 (0)	0/12 (0)
Linezolid, 25 (12)	4.5 (4.1–5.0)	7.3 (7.1–7.7)	1/12 (8)	0/12 (0)
Linezolid, 50 (12)	5.1 (4.1–6.0)	7.1 (6.8–8.0)	2/12 (17)	0/12 (0)
Linezolid, 75 (12)	4.6 (3.2–5.0)	4.5 (4.0–5.2)	3/12 (25)	2/12 (17)
Rifampin, 12.5 (12)	3.1 (3.0–3.4)	<3.0	6/12 (50)	11/12 (92)
Linezolid, 25, + rifampin, 12.5 (20)	<3.0	<3.0	13/20 (65)	19/20 (95)
Linezolid, 50, + rifampin, 12.5 (20)	<3.0	<3.0	13/20 (65)	15/20 (75)
Linezolid, 75, + rifampin, 12.5 (20)	<3.0	<3.0	11/20 (55)	17/20 (85)
Levofloxacin, 10, + rifampin, 12.5 (24)	<3.0	<3.0	18/24 (75)	11/11 (100)

[a] Values are medians (interquartile ranges).
[b] Values in parentheses in this column are the number of cages.

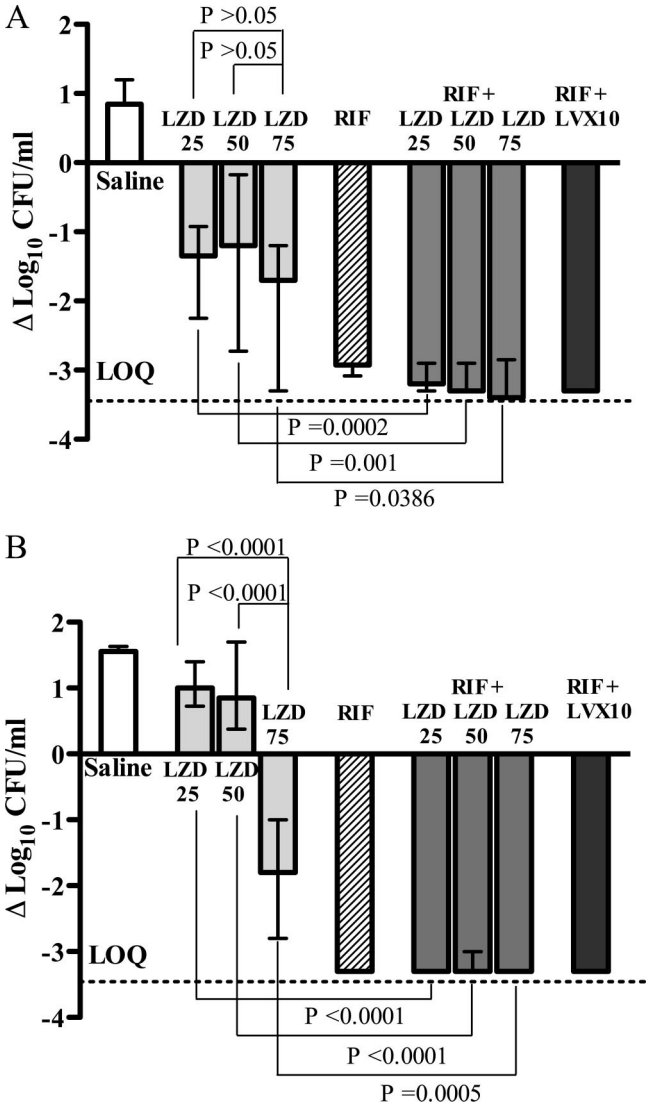

FIG. 3. Efficacy of treatment against planktonic bacteria in cage fluid ($\Delta\log_{10}$ CFU/ml) during treatment (day 4) (A) and 5 days after the end of treatment (day 10) (B). The dashed horizontal line indicates the limit of quantification (LOQ). LZD25, linezolid at 25 mg/kg; LZD50, linezolid at 50 mg/kg; LZD75, linezolid at 75 mg/kg; RIF, rifampin at 12.5 mg/kg; and LVX10, levofloxacin at 10 mg/kg.

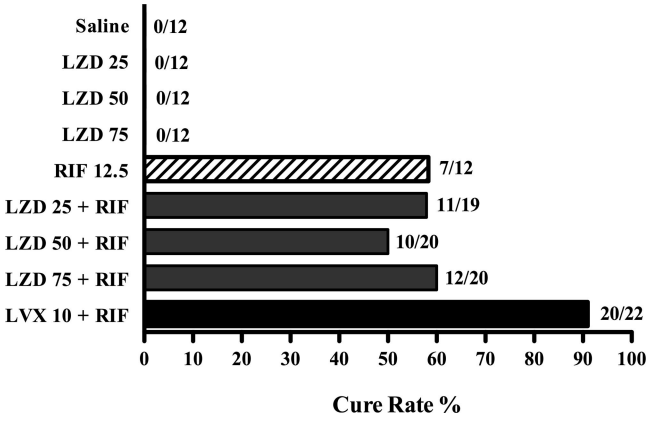

FIG. 4. Cure rates of cage-associated infection at day 10. The values indicate the number of cage cultures without growth of MRSA/total number of cages in the treatment group. LZD25, linezolid at 25 mg/kg; LZD50, linezolid at 50 mg/kg; LZD75, linezolid at 75 mg/kg; RIF, rifampin at 12.5 mg/kg; and LVX10, levofloxacin at 10 mg/kg.

organism was inhibited by linezolid at 2.5 μg/ml. However, a reduction of ≥99.9% CFU/ml was not achieved at concentrations up to 20 μg/ml in either the logarithmic or the stationary growth phase. This is in agreement with the bacteriostatic activity of linezolid against staphylococci (13). On the basis of this characteristic, linezolid monotherapy does not seem to be appropriate for the treatment of staphylococcal implant-associated infections.

In the in vitro time kill-curve studies, rifampin resistance was detected after 24 h of incubation in all cultures exposed to rifampin alone (1× and 4× the MIC). In contrast, the use of rifampin in combination with linezolid never resulted in the emergence of rifampin resistance (11, 12, 29). Thus, we can conclude that in vitro the combination linezolid-rifampin did not display any synergism or antagonism against the strain tested, and it was difficult to interpret whether there was any additive effect because of bacterial regrowth at 24 h of incubation with rifampin alone. However, the combination was effective in completely preventing the development of rifampin resistance.

In the pharmacokinetic studies, the peak linezolid concentrations in cage fluid increased linearly with increasing doses between 25 and 75 mg/kg, whereas the increase in the AUC_{0-24} was not proportional to the dose due to the faster elimination of linezolid from the cage fluid at higher doses. The peak linezolid concentration and the AUC_{0-24} reached in the cage fluid with the 75-mg/kg dose were comparable to the values reported by Gee et al. (10) in the inflammatory blister fluid of healthy volunteers receiving 600 mg linezolid every 12 h. The 25-mg/kg and the 50-mg/kg linezolid doses administered to guinea pigs more likely simulate the doses achieved with the 400-mg and 600-mg single-dose regimens, respectively. The three doses of linezolid chosen guaranteed that the antimicrobial concentration in cage fluid remained above its MIC for the test organism (2.5 μg/ml) for 12 h and, thus, during the entire treatment. Andes et al. (1a) showed that a plasma AUC_{0-24}/MIC ratio of linezolid between 50 and 100 was predictive of a successful outcome of staphylococcal infections in the thigh muscle model. In our studies, the AUC_{0-24}/MIC was only approximately 50 and was achieved with the highest linezolid dose (75 mg/kg). However, these values are difficult to interpret since we investigated a different compartment (cage fluid) and a different type of infection (an infection associated with an implant) compared to those used by Andes et al. (1a).

The rifampin dose of 12.5 mg/kg was chosen as described previously (31, 34). The peak levels in tissue fluid were equal to or less than the maximal concentrations reached in humans (1), and the rifampin concentration in cage fluid was greater than the MIC during 12 h after administration.

The cage fluid from the MRSA-infected cages implanted in guinea pigs demonstrated continuous bacterial growth for 10 days and no spontaneous cure. Linezolid induced a significant reduction in the counts of planktonic bacteria during treatment (day 4) both when it was given alone and when it was given in

combination with rifampin ($P < 0.05$) (Fig. 3A). During treatment, no difference between the linezolid monotherapies was observed, but in combination with rifampin, bacterial killing was significantly improved ($P < 0.05$). Five days after the end of treatment (day 10), bacterial regrowth occurred with linezolid doses of 25 and 50 mg/kg, whereas the counts remained suppressed after the linezolid dose of 75 mg/kg (Fig. 3B), even though the $AUC_{0\text{-}24}$ was only slightly higher (Table 2). As shown previously (20), linezolid is able to induce a postantibiotic effect in *S. aureus* in a dose-dependent manner in vitro. Thus, it is likely that the postantibiotic effect was induced by the highest linezolid dose (75 mg/kg) but not by the two lower doses. In addition, the accumulation of linezolid may have occurred with the highest dose, delaying its time of clearance from the cage fluid. All combinations of linezolid with rifampin inhibited bacterial regrowth 5 days after administration of the last dose.

None of the treatment regimens with linezolid monotherapies eradicated the cage-associated MRSA infections, while the combinations of linezolid with rifampin achieved cure rates of between 50% and 60%, which is not significantly different from that achieved with rifampin monotherapy. The combination of rifampin and levofloxacin showed the highest cure rate (91%). Treatment failures were related to a lack of efficacy in the killing of bacteria when they were embedded in the biofilm matrix. The emergence of rifampin resistance did not occur in vivo with any of the rifampin regimens tested.

In conclusion, linezolid monotherapies showed bacteriostatic activity against the MRSA strain tested and were not able to eradicate the adhering bacteria. Thus, linezolid should not be used alone for the eradication of implant-associated infections caused by MRSA. In vitro studies demonstrated the potential of the linezolid-rifampin combination for the treatment of MRSA infections, and these findings were confirmed in the animal foreign-body infection model. However, levofloxacin-rifampin combinations achieved higher cure rates than the linezolid-rifampin combination against the quinolone-susceptible MRSA strain tested (91% and 50 to 60%, respectively). In contrast to our previous recommendations (41), the quinolone-rifampin combination seems to be a valid option for the treatment of MRSA infections, whereas linezolid-rifampin regimens may be used for the treatment of quinolone-resistant MRSA implant-associated infections.

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